POLAROGRAPHIC DETERMINATION OF EQUILIBRIUM CONSTANTS

by

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ABSTRACT

Polarographic limiting currents make it possible to study slowly established equilibria occurring in the area of the dropping mercury electrode.

It was the purpose of this project to graphically determine the pK value for the pyruvic acid-glycine reaction by studying the shape and slope of the polarographic wave. Once an accurate value for this reaction was obtained, the reactions of citric, fumaric, and malic acids with glycine and pyruvic acid with alanine were investigated.

GENERAL THEORY

Voltammetry is a general term applied to the branch of electroanalytical chemistry dealing with the effect of the potential of an electrode in an electrolysis cell.[1] Polarography is that branch of voltammetry dealing with the determination of the current versus voltage curve in a system. Although various electrodes can be used, the dropping mercury electrode was used in this study.

In polarography, the area of the drop formed by the electrode is small, and the current flowing through the cell is also very small. Therefore, the IR drop through the cell can easily be made small, and then the voltage impressed across the two electrodes will be virtually equal to the difference between their potentials.[2] By connencting the indicator electrode to a recording device, the resulting potential is charted as a polarographic wave. The plateau of the polarographic wave represents the total limiting current flowing through the cell at a particular potential. This limiting current of the polarographically active substance is the sum of three components.

The first component is the residual current or "the current that would flow under the same conditions but in the absence of the substance responsible for the wave."[3] The residual

current is, in turn, composed of two factors. The fraction of residual current due to the reduction of small concentrations of impurities such as heavy metal ions in the supporting electrolyte is refered to as the faradaic factor as it obeys Faraday's laws of electrolysis.[4] The other component of residual current is condenser current. It results from the electric double layer that is created around each drop of mercury because of the specific charge on the drop. If, for example, the drop is negatively charged, a positively charged layer will then accumulate very near the surface of the mercury drop. This positively charged layer will then be surrounded by a negatively charged layer of molecules. "The current required to charge and maintain the electrical double layer is accordingly known as the condenser current."[5] (The magnitude of the condenser current is dependent on the potential of the dropping mercury electrode.)

The second component of the limiting current is the migration current, the difference between the limiting current actually obtained and the limiting current that would be obtained in the absence of any electrostatic force.[6] An electrostatic force of either attraction or repulsion exists between the molecules of the substance responsible for the wave and the electrode. It is this force that affects the rate at which the molecules reach the electrode surface and undergo reduction

or oxidation.

The third component of limiting current and the most important constituent is the diffusion current. Diffusion current "reflects the rate at which the ions or molecules responsible for the wave reach the electrode surface under the sole influence of a diffusive force."[7] The occurrence of the diffusive force is a result of the concentration gradient between the bulk of the solution and the area adjacent to the electrode. As soon as molecules reach the surface of the electrode, they are immediately reduced or oxidized. Therefore, the concentration of these molecules in the layer of solution around the electrode is essentially zero.

The size of the diffusion current is given by the Ilkovič equation,

$$i_d = .627\, n F D^{1/2} m^{2/3} t^{1/6} \cdot C$$

In this equation n, the number of electrons transfered, is constant for any one electrode system. F, the faraday, is the charge and will also remain constant in any one system. D, the diffusion coefficient, expresses the rate of diffusion of the particular substance being studied. The term D is greatly affected by temperature. A 1°C rise in temperature causes a 1.5% increase in the height of the diffusion current.[8] The outflow of mercury, m, and the dropping time of the electrode, t, are collectively referred to as the capillary characteristics.

When a constant temperature is maintained and when the outflow of mercury and the dropping time of the capillary stay the same, a linear relationship exists between the diffusion current and the concentration. As long as these restrictions are met, the Ilkovic equation may be written $i_d = Kc$. Therefore, by holding the constituents of factor K constant, a direct comparison of resulting polarographic curves may be made.

By measuring these limiting currents, it is possible to ascertain equilibrium constants when the equilibrium is established slowly in comparison with the dropping time of the electrode.[9]

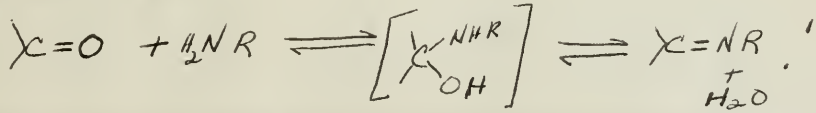
$$D + Y \rightleftharpoons E$$

Generally, the time necessary for the establishment of equilibrium is five to ten times greater than the dropping time of the electrode. "For polarographic determination of the equilibrium constants, it is necessary that substances D and E are reduced at different potentials, in separate waves such that at least one, (D or E) but preferably both, are reduced in the potential range available."[10]

Furthermore, conditions are favorable for polarographic determination when equilibrium is shifted to the left, in favor of the reactant D--the product E being formed in concentrations that are only a fraction of the concentration of D.[11] It is therefore necessary that substance Y be present

in excess and be electro-inactive in the potential range studied. When the concentration of Y needed for equilibrium is greater than ten times the concentration of D, it is possible to obtain the equilibrium constant from the value of the concentration of Y for which $i_D = i_E$. This is accomplished graphically by plotting the log $i/i_d - i$ against the log of the concentration of Y. The dependence should be linear and the value of -log of the concentration of Y at the point coresponding to the zero value of the logarithm of the current, equals numerically the pK value.[12]

According to Zuman's theory, the graphic determination of pK values is limited to reactions involving carbonyl compounds (as D) and primary amines (as Y). The general mechanism here is:



[13]

The amine group undergoes nucleophilic addition to the carbonyl compound due to the free electron pair of the unprotonated form of the amine. The reaction goes through an intermediate enol and then, by eliminating water, produces an imine.

Each polarogram run using a carbonyl compound and a primary amine produces at least two plateaus per wave. The

more negative wave is associated with the reduction of the carbonyl compound. The wave at the more positive potential corresponds to the reduction of the imine, $>C=NR$. The height of the more positive wave increases proportionately with increasing concentrations of the amine.[14]

The system is extremely sensitive to pH and the reaction must be run in a well buffered media. The effective pH range is 8.3-11.0 and it is impossible to follow the behavior of the imine product over a wider range.[14] Specifically, studies aimed at determining the equilibrium constants involving imine products must be run in a buffer having a pH of plus or minus one pH unit of the value where pH = pK_a of the amine.[14]

INSTRUMENTATION

To measure polarographic currents, it is necessary to have a dropping mercury electrode and a reference electrode placed in an electrolysis cell with the appropriate circuit connections.

The dropping mercury electrode used here was a commercial five inch piece of thick walled capillary with a small internal diameter and a dropping time of five seconds (5.0). One end of the capillary was immersed in the solution being analyzed and the other end was attached (by low sulfur content tubing) to the mercury reservoir--54cm above the electrode. Therefore, formation of the droplet depended on the interfacial tension between the mercury and the solution and the geometry of the capillary tip.

The polarographic cell was a standard Lingane-Laitinen H-cell. There were two compartments, one containing the solution being analyzed and the other containing the reference electrode. The compartments were separated by a cross member filled with a 4% agar-saturated potassium chloride gel which was held in place by a medium porosity sinterred Pyrex disc. The disc was located as near the solution compartment as possible and the side tube through which inert gas was passed was as near the bottom as possible. This arrangement facilitated rapid and complete deaeration of the solution. The agar gel

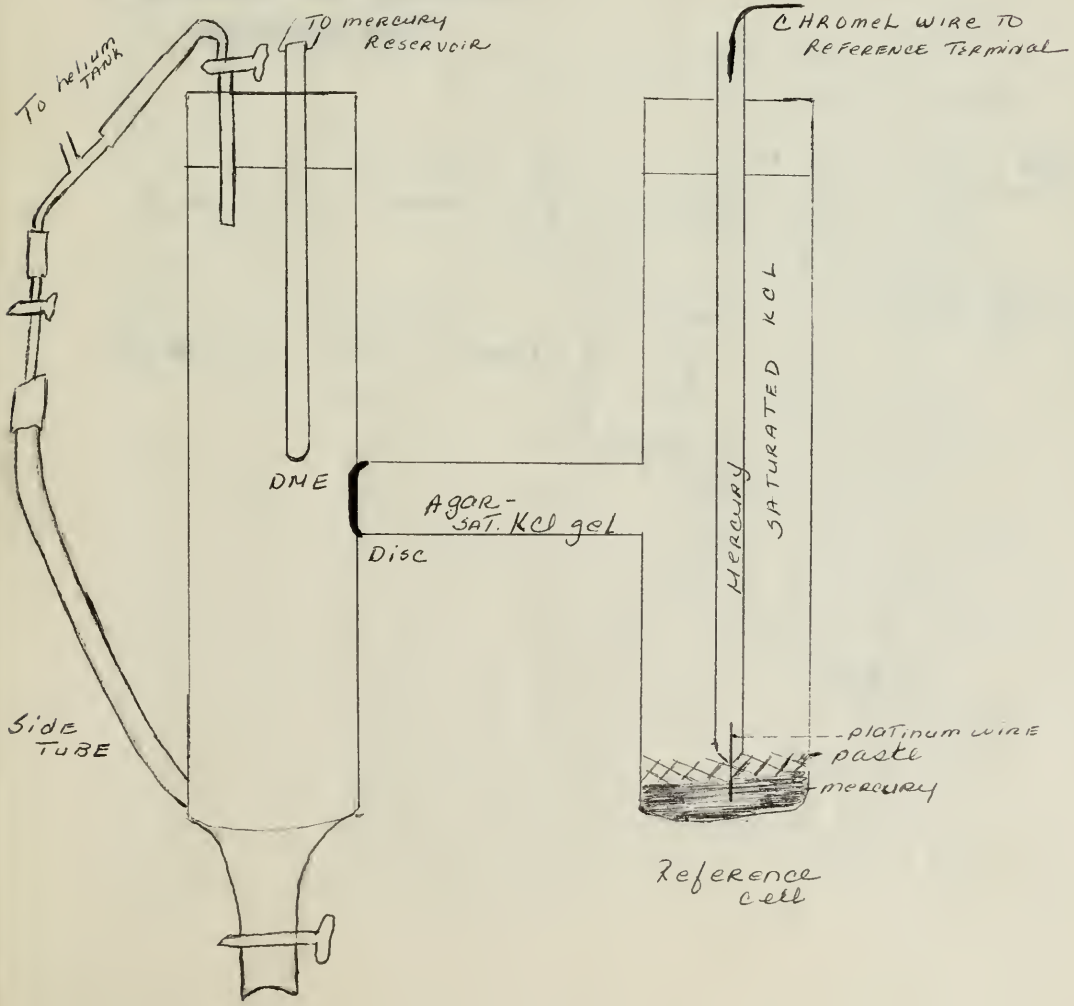
was prepared by warming four grams of high grade agar, 30 grams of potassium chloride and 90ml of water over a steam bath. When the gel liquified, it was poured into the bridge and allowed to solidify.[15]

Next, the saturated calomel reference electrode was constructed. (The saturated calomel electrode was chosen instead of a mercury pool or silver chloride electrode because the S.C.E. provides for an external reference system and its potential is independent of the composition of the sample solution.) To make the S.C.E. about three centimeters of distilled mercury was poured into the compartment. A paste of mercurous and potassium chlorides (mixed to paste form by adding a few drops of saturated potassium chloride) was then added until it was approximately the same height as the mercury. The compartment was then filled with saturated potassium chloride. Electrical contact was secured by running a platinum wire down a glass tube such that the wire projected into the mercury. The glass is melted around the tip until a tight seal is formed and then mercury is poured into the tube. A chromel wire leading to the reference terminal of the circuit was then inserted in the mercury (in the tube).

The solution compartment was filled with a saturated potassium chloride solution to keep the agar gel from drying out. In practice, the solution compartment was thoroughly washed and rinsed with the sample solution. Before running

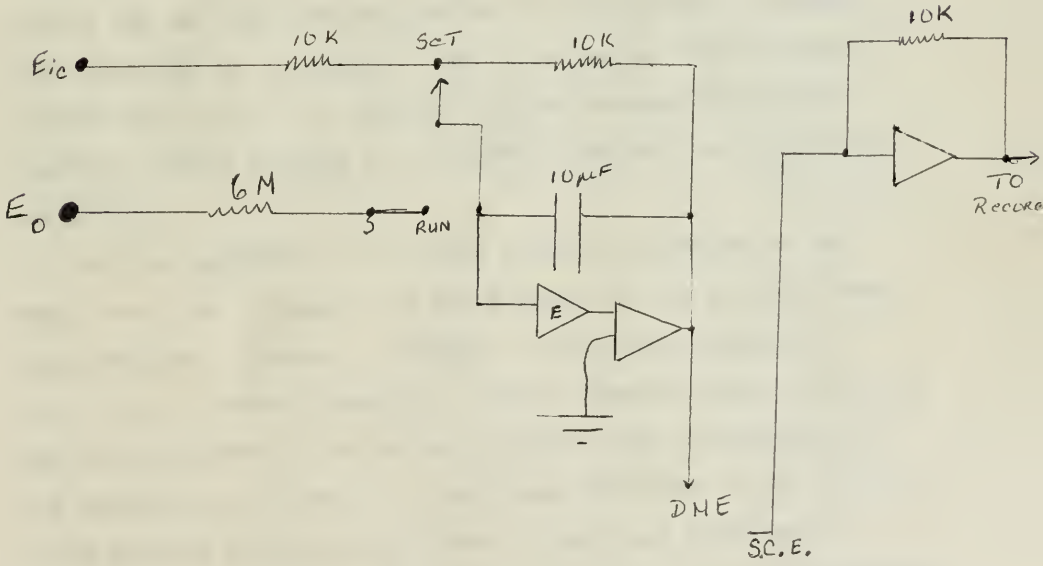
the polarogram, dissolved oxygen was removed to eliminate the characteristic oxygen wave. This was accomplished by deaeration with helium for ten minutes. Once deaeration was completed, the gas was diverted over the surface of the solution.

The completed cell resembled the following diagram:[16]



The basic console for the polarographic circuit was the McKee-Pedersen--1001. For this circuit, the operational amplifier (MP--1006), the electrometer/operational amplifier (MP--1032), the intergrator (MP--1012) and the recorder (MP--1027) were used. The resistors and wires were also supplied by McKee-Pedersen.

The circuit was[17]



The polarograph is composed of three parts: a source of e.m.f. enabling a continuous change of voltage to the electrolysis cell, an indicator showing the current flowing through the cell during electrolysis, and an arrangement enabling the recording of the current indicated, as a function of the applied voltage.[18]

The input resistor was six megohms which gave a time constant, RC, of ($6 \times 10^{6} \cdot 10 \times 10^{-6}$) 60 seconds. Therefore, when a 100 mv ramp was applied to the integrator, the ramp rose linearly at 100mv/min. And by using an electrometer, essentially all of the current flowing through the input resistor passed through the feedback loop and not into the amplifier.

As the potential of the DME changed according to the ramp rate, the molecules on the surface of the mercury drop were reduced. (Hence, a cathodic current was measured.) Due to the electron transfer, current flowed through the cell and the reduction of solution molecules was accompanied by the oxidation of mercury to mercurous chloride in the S.C.E. (The current flowing under these conditions depended on two factors: the ratio of the concentrations of the unreduced to reduced substances which prevailed at the surface of the electrode to satisfy the Nernst equation at the specific potential applied and the rate at which the substance being

reduced reached the electrode surface by the diffusion process.[19] The second factor was proportional to the concentration of the polarographically active substance in the bulk of the solution, to the area of the electrode, and depended on the mobility of the molecules being reduced.[20])

The S.C.E. was connected directly to a current sensitive operational amplifier which was at virtual ground. The amplifier is a nearly ideal measuring device because it has a low input impedance. The output of the current sensitive amplifier circuit is given by $E_0 = Z_f \cdot i_s$. (Z_f was chosen to be 10K, since the output is limited to one volt and the current could be as high as 100 microamperes.) And the output is connected to a potentiometric recorder, where polarographic curves were charted.

RESULTS

The pyruvic acid-glycine reaction was the first system investigated. This reaction was chosen because much information is available about the necessary experimental conditions as well as the polarographically determined pK value for the reaction. By duplicating the work previously done by Zuman[14], it was possible to evaluate the accuracy of the method.

Three essential factors must be considered: the $D + V \rightleftarrows E$ equilibrium is established slowly, the diffusion current is controlled, and there is a linear relationship between the diffusion current and the concentration. The rate of equilibrium is governed by the pH of the solution, and equilibrium is established slowly in an alkaline solution.[14] The K_a of glycine is 1.6×10^{-10} thus, the $pH=pK_a$ is 9.8. Specifically, a pH of 9.2 was chosen.

The buffer solution is also the supporting electrolyte for the system. Besides providing the conditions for the best waves, the supporting electrolyte maintains the conductivity of the solution and eliminates the effect of migration currents. To eliminate migration effects, a .1M buffer solution and a 5.6×10^{-4} M stock solution of pyruvic acid were used. (When the concentration of the supporting electrolyte is twenty times the concentration of the substance being studied, the migration effects are excluded.)

The limiting current was corrected for residual current by geometrical subtraction of the residual current registered separately in the supporting electrolyte. A residual current of 5.0×10^{-6} amperes was observed.

Finally, to ensure a linear relationship between the diffusion current and the concentration, the following criteria were established: the dropping time of the electrode was 5.0 seconds and the mercury flow was 2.3 mg/sec. Temperature was constant.

Pyruvic acid proved to be polarographically active; glycine was not sensitive. The reacting mixture of these two substances consisted of 20ml of glycine (in varying concentrations of .05M, .10M, .30M, .50M, .75M, 1.25M, and 1.75M), 2ml of 5.6×10^{-4}M pyruvic acid and 1ml of 2% Triton X-100 to suppress the maxima. (Maxima are seen as a sharp rise in the current and are accompanied by motion of the solution around the electrode. This movement causes an increased transport of the electroactive species to the surface of the DME.)

Excellent polarograms resulted, showing the two expected waves--the first of which increased with increasing concentrations of the glycine. Making calculations, a pK value of 2.46 was determined. This is in close agreement with the value determined by Zuman, 2.47.[14]

Using the similarity of the known value as an indicator of the accuracy of the method, citric, fumaric, and malic acid-glycine systems were investigated. These reactions had not previously been examined.

All experimental conditions remained the same. Citric acid was made up to 5.6×10^{-4}M and was polarographically active. The resulting polarograms showed three waves. The most positive wave increased with increasing concentrations of glycine, indicating the reduction of the imine. The second wave was the result of the reduction of the carbonyl group; the specific C=O group being reduced was not determined. The third wave did not give reproducible results when the solutions were run a second and third time. (This may have been due to to the buffer needed to study this wave or to the development of a kinetic current.) Making calculations based on the first two waves, a pK value of 3.78 was obtained for the reaction.

Fumaric acid was then examined. Experimental conditions were the same. A 5.6×10^{-4}M solution of fumaric acid proved to be polarographically active. Two distinct waves were observed with glycine as the amine and a pK value of 1.96 was calculated.

With all the factors remaining constant, a 5.6×10^{-4} M stock solution of malic acid which was polarographically active yielded a pK value of 4.1.

It was then decided to consider the reaction of pyruvic

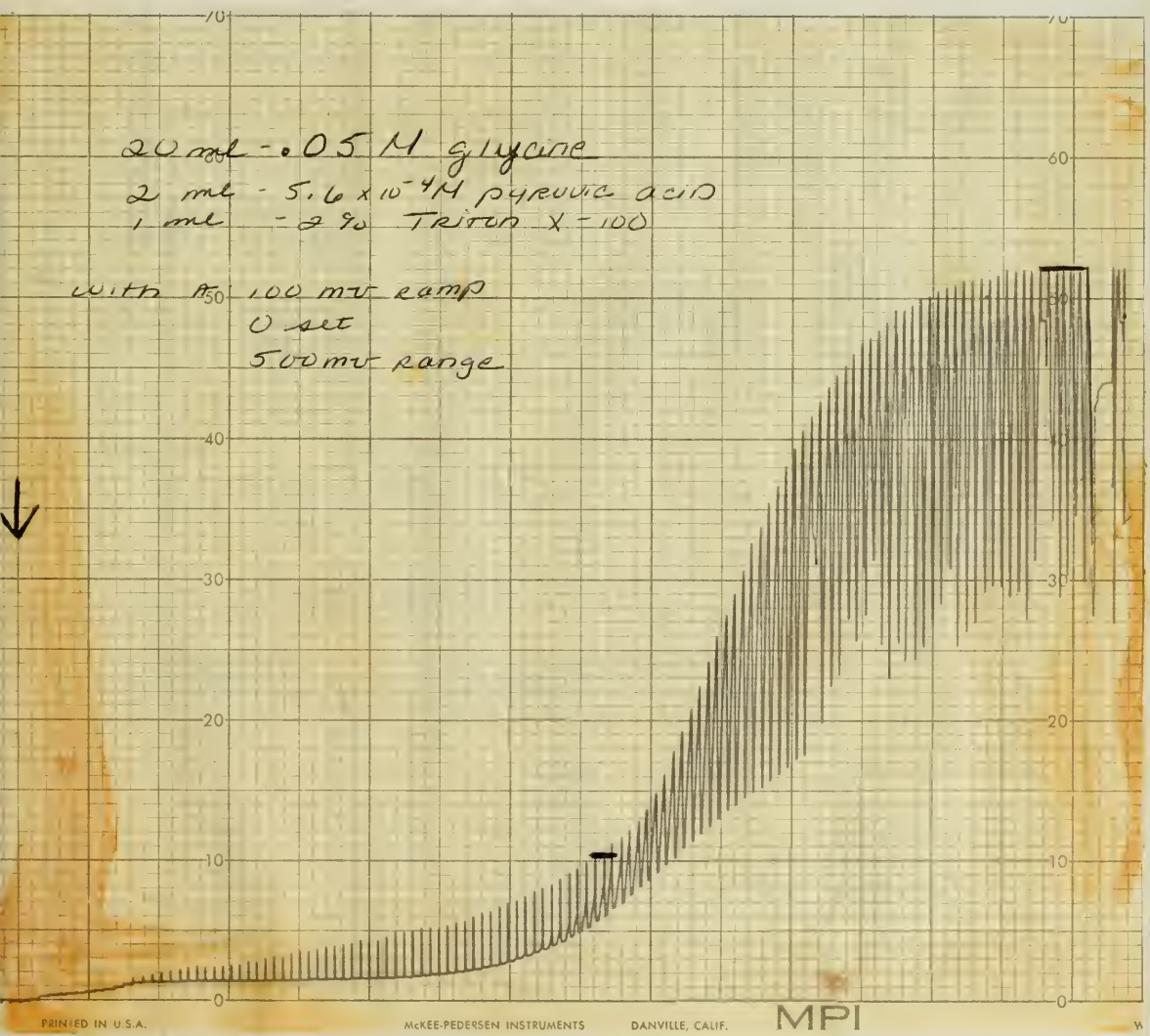
acid with another amine. Alanine was selected because it is similar to glycine in that it is polarographically inactive and it requires the same buffer. All factors constant, a pK value of 0.92 was obtained. (It was noted that .05M and .10M solutions of alanine failed to produce any waves. This may be the result of the formation of another product other than the imine at these concentrations and under the influence of the 9.2 buffer.) The accurate reproducibility of the method has again been illustrated because Zuman's pK value for the pyruvic acid-alanine system is 0.93.[14]

From these results it is obvious that this application of organic polarography is precise and sensitive. It is relatively simple to operate and offers a variety of applications in the study of equilibria.

APPENDIX INDEX

MAKING CALCULATIONS

The curve below is an example of the polarograms obtained in this project.



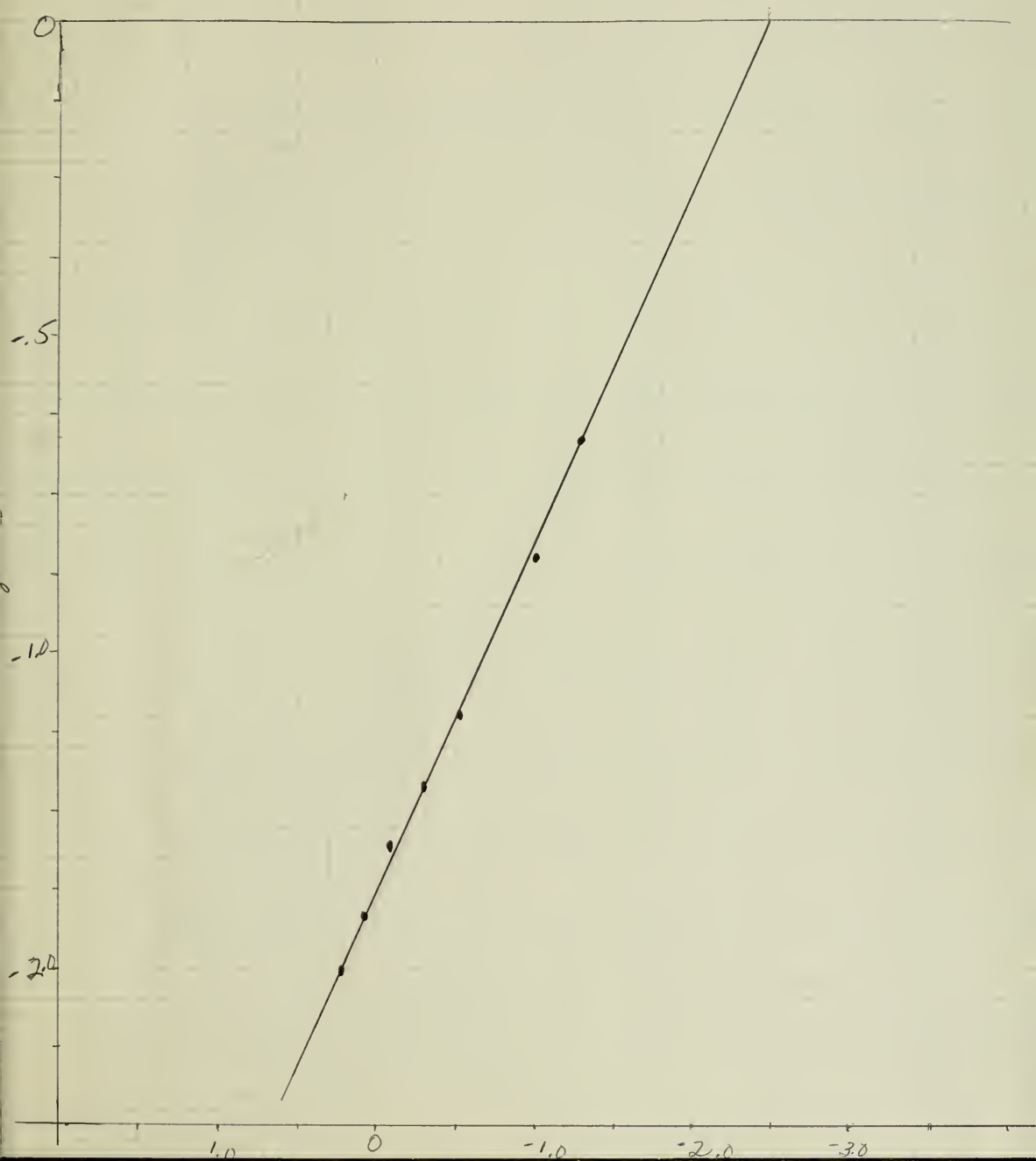
From the polarogram, the value of i, the diffusion current of the more positive wave, and i_d, the total diffusion current, may be computed for one concentration of glycine. (Plotting the log $i/i_d - i$ against the log of the concentration of glycine for the various concentrations of glycine studied yields a straight line. And the value of -log of the concentration of glycine corresponding to the zero value of the log of the current numerically equals the pK.)

Range = 500mv
Residual current = 5.0×10^{-6}amps.

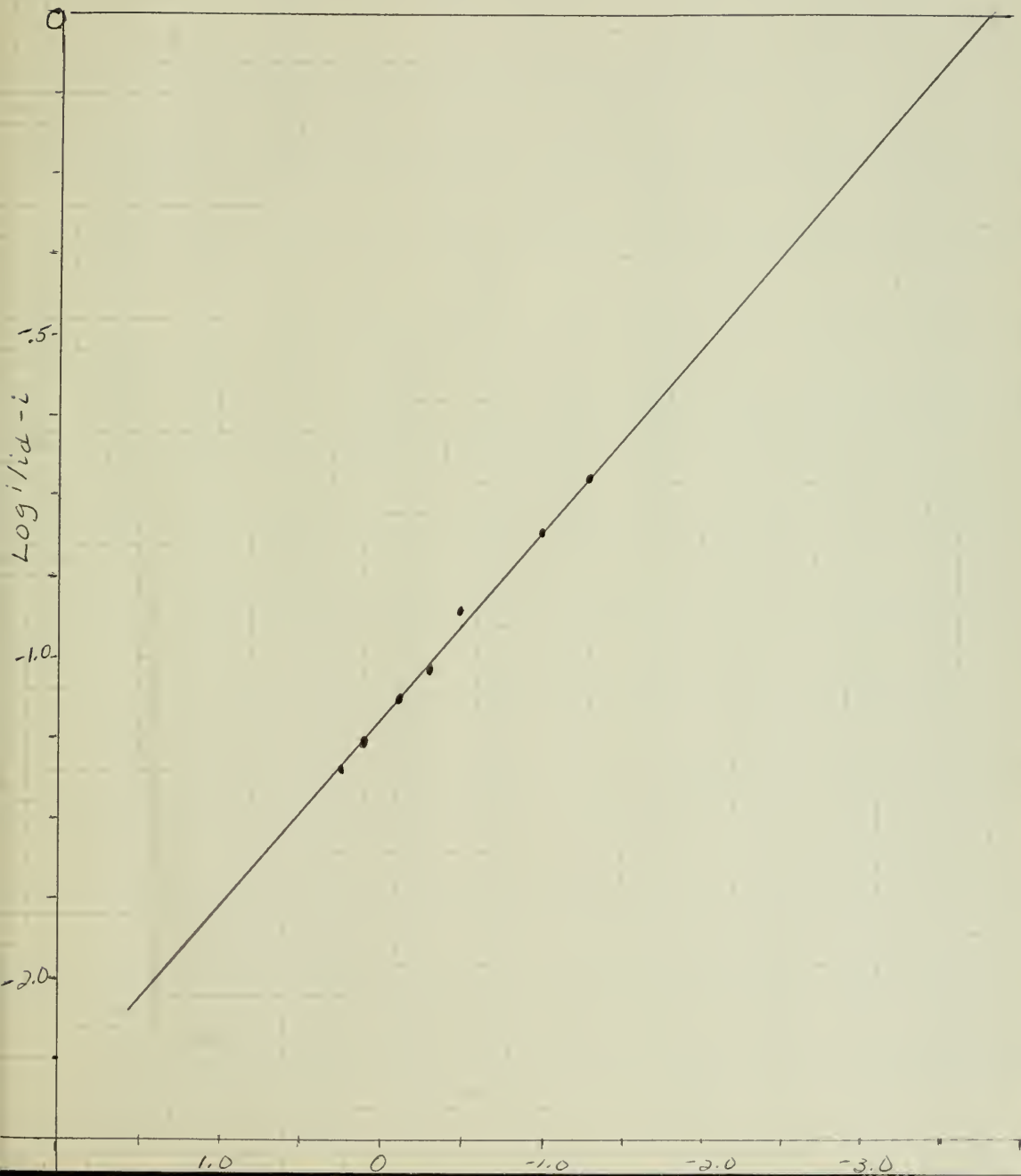
i	i_d	i/i_d-i	log i/i_d-i
E=(10mv)(5mv)	E=(52mv)(5)	$\frac{45 \times 10^{-6}}{210 \times 10^{-6}}$	=-0.6696
$= 50 \times 10^{-2}$V	$= 260 \times 10^{-2}$V	$= 2.14 \times 10^{-1}$	
R=1×10^{-4}ohms	R= 1×10^{-4}		
i= E/R	i= E/R		
=50×10^{-6}amps	=260×10^{-6}		
-5×10^{-6}amps	$- 5 \times 10^{-6}$		
i= 45×10^{-6}amps	i_d=255×10^{-6}		

CONCENTRATION OF GLYCINE	LOG CONCENTRATION OF GLYCINE
.05M	-1.3010
.10M	-1.0000
.30M	-0.5229
.50M	-0.3110
.75M	-0.1249
1.25M	+0.0969
1.75M	+0.2430

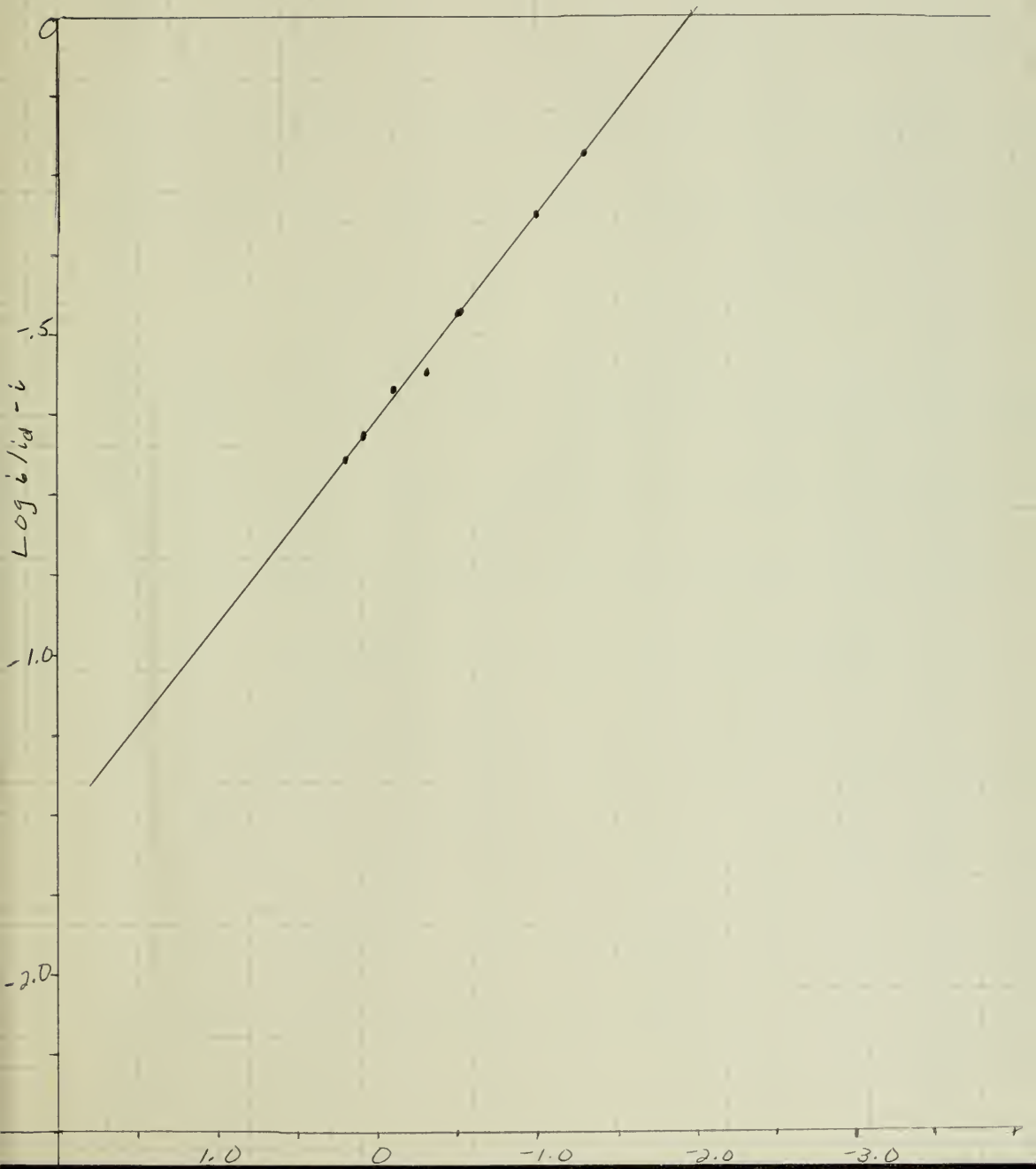
pK determination for the pyruvic acid-glycine reaction



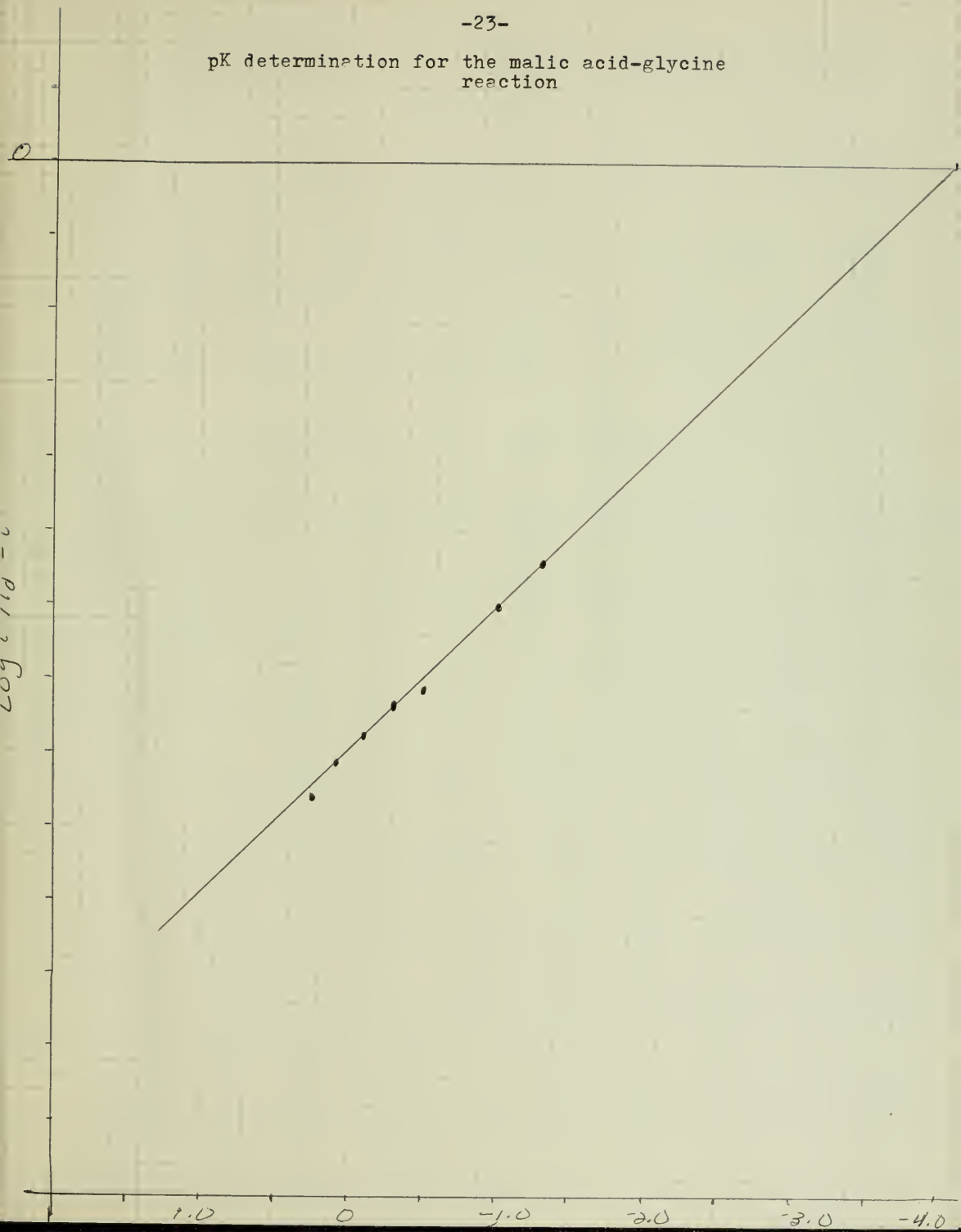
pK determination for the citric acid-glycine reaction



pK determination for the fumaric acid-glycine reaction



pK determination for the malic acid-glycine reaction



pK determination for the pyruvic acid-alanine reaction

SUMMARY OF EXPERIMENTAL CONDITIONS

Polarizing voltage: 100mv ramp

Capillary characteristics: Dropping time = 5.0 sec.
Mercury flow = 2.3 mg/sec

Height of mercury column: 54 cm

Concentration of carbonyl substances: 5.6×10^{-4} M
(Note: These acids must be kept refrigerated to avoid growth of bacteria.)

Concentrations of amines: .05M, .10M, .30M, .50M, .75M, 1.25M, and 1.75M

(Note: These solutions must be four to six hours old.)

Reacting mixture: 20ml of the amine, 2ml of the acid, and 1ml of detergent.

Deaeration: 10 minutes with helium

Buffer: pH=9.2

CAPILLARY CARE

There are numerous techniques available in polarographic literature for the cleaning and storing of capillaries. The most successful proceedure used here included several steps. The capillary was thoroughly washed with 100ml of acetone and then 100ml of distilled water. It was allowed to dry with the mercury flowing. Once dried, the capillary was raised 40cm (to the point where the mercury stopped flowing) and stored in a clean dry test tube.

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